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| (54) Title: CYCLIC PROSAPOSIN-DERIVED PEPTIDES AND USES THEREOF | | |
| (57) Abstract Cyclic neurotrophic and analgesic peptides derived from the active region of prosaposin having the consensus sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$ (SEQ ID NO:4), wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. The peptides are useful in the promotion of neural outgrowth, prevention of cell death, promotion of myelination, treatment of neurodegenerative disorders and treatment and prevention of neuropathic pain. | | |

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CYCLIC PROSAPOSIN-DERIVED PEPTIDES AND USES THEREOF

Field of the Invention

5 The present invention relates to neurotrophic and analgesic peptides. More particularly, the invention relates to cyclic peptides derived from the active region of saposin C which have neurotrophic and analgesic effects.

Background of the Invention

10 Neurotrophic factors are proteins or peptides capable of affecting the survival, target innervation and/or function of neuronal cell populations (Barde, *Neuron* 2:1525-1534, 1989). The efficacy of neurotrophic factors both *in vitro* and *in vivo* have been well-documented. For example, nerve growth factor (NGF) acts as a trophic factor for forebrain cholinergic, peripheral and sensory neurons (Hefti et al., *Neurobiol. Aging* 10:515-533, 1989) and can reverse naturally-occurring as well as physical traumatic injuries to peripheral nerves (Rich et al., *J. Neurocytol.* 16:261-268, 1987). Brain-
15 derived neurotrophic factor (BDNF) is a trophic factor for peripheral sensory neurons, dopaminergic neurons of the substantia nigra, central cholinergic neurons and retinal ganglia (Henderson et al., *Restor. Neurol. Neurosci.* 5:15-28, 1993). BDNF has been shown to prevent naturally-occurring cell death both *in vitro* and *in vivo* (Hofer et al., *Nature* 331:262-262, 1988). Ciliary neurotrophic factor (CNTF) promotes survival of
20 chicken embryo ciliary ganglia *in vitro* and supports survival of cultured sympathetic sensory and spinal motor neurons (Ip et al., *J. Physiol. Paris* 85:122-130, 1991). Demyelination is a defect common to a number of central nervous system (CNS) disorders, the most prevalent being multiple sclerosis (MS). MS, a chronic disorder which may lead to total disability, is characterized by damage to the myelin sheath, while leaving the axons
25 mostly intact. There is currently no effective treatment for MS. Other central nervous system disorders involving demyelination include acute disseminated encephalomyelitis, amyotrophic lateral sclerosis, acute hemorrhagic leukodystrophy, progressive multifocal leukoencephalitis, metachromatic leukodystrophy and adrenal leukodystrophy. The peripheral nervous system (PNS) can also be afflicted with demyelination, such as that occurring in Guillain-Barré
30 syndrome (*Pathologic Basis of Disease*, Robbins et al. eds., W.B. Saunders, Philadelphia, 1979, pp. 1578-1582).

Peripheral nerve injuries and peripheral neuropathies, such as those resulting from diabetes or chemotherapy, comprise the most prevalent peripheral nervous system disorders. Current treatments for peripheral nervous system disorders only treat the symptoms, not the cause of the disease.

5 Neuropathic pain results from nerve injury, such as a nerve compression or crush and traumatic injury to the spinal cord, and is often long-lasting or chronic. Most traumatic nerve injuries also cause the formation of neuromas, in which pain occurs as a result of aberrant nerve regeneration. In addition, cancer-related neuropathic pain results when tumor growth compresses adjacent nerves, brain or spinal cord. Neuropathic pain
10 is also associated with diseases including diabetes and alcoholism. In most cases, neuropathic pain is resistant to current drugs. These drugs also have serious side-effects. U.S. Patent Application Serial No. 08/611,307 provides a method for alleviating or preventing neuropathic pain by administering to an individual an effective amount of an active fragment of prosaposin.

15 Prosaposin is the precursor of a group of four small heat-stable glycoproteins which are required for hydrolysis of glycosphingolipids by lysosomal hydrolases (Kishimoto et al., *J. Lipid Res.* 33:1255-1267, 1992). Prosaposin is proteolytically processed in lysosomes, generating saposins A, B, C and D (O'Brien et al., *FASEB J.* 5:301-308, 1991). O'Brien et al. (*Proc. Natl. Acad. Sci. U.S.A.* 91:9593-9596, 1994),
20 U.S. Patent No. 5,571,787 and published PCT Application No. WO95/03821 disclose that prosaposin, saposin C stimulate neurite outgrowth and promote increased myelination. In addition, U.S. Patent Nos. 5,571,787, 5,696,080, 5,714,459 and published PCT application No. WO95/03821 disclose that a 22-mer peptide (CEFLVKEVTKLIDNNKTEKEIL; SEQ ID NO: 1) consisting of amino acids 8-29 of
25 human saposin C stimulates neurite outgrowth. These references also disclose that an 18-mer peptide (YKEVTKLIDNNKTEKEIL; SEQ ID NO: 2) contained within the active 22-mer of saposin C (with V replaced by Y) also promotes neurite outgrowth and is capable of crossing the blood brain barrier. O'Brien et al. (*FASEB J.* 9:681-685, 1995) show that the 22-mer stimulates choline acetyltransferase activity and prevented
30 neuronal cell death. The active neuritogenic fragment was localized to a linear 12-mer sequence located within the amino terminus of saposin C (LIDNNKTEKEIL; SEQ ID

NO: 3) (O'Brien et al., *FASEB J.* 9:681-685, 1994). Liepinsh et al. (*Nature Struct. Biol.* 4:793-795) indicated that the loop conformation of a neurotrophically active peptide segment of NK-lysin (residues 17-30) could be mimicked by a circular peptide.

A major obstacle to the *in vivo* therapeutic use of peptides is their susceptibility to proteolytic degradation. The present invention provides cyclic prosaposin peptidomimetics which have good resistance to proteolytic degradation and are capable of crossing the blood brain barrier.

Summary of the Invention

One embodiment of the present invention is a cyclic neurotrophic and analgesic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (alanine, leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. Preferably, the peptide has the amino acid sequence shown in SEQ ID NO: 5 or 6.

The present invention also provides a composition comprising the peptide described above in a septum sealed vial, formulated with a controlled release material, in lyophilized form, in liposomal form, in a form suitable for topical administration or in unit dosage form.

Another embodiment of the present invention is a method for inducing myelination or inhibiting demyelination in a mammal, comprising administering to a mammal afflicted with demyelination a pharmaceutically effective demyelination inhibiting amount of a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. In one aspect of this preferred embodiment, the demyelination is due to multiple sclerosis, ischemic injury or traumatic injury. Preferably, the administration is

intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal or topical. Advantageously, the peptide is administered in a pharmaceutically acceptable carrier. In another aspect of this preferred embodiment, the peptide is enclosed in a lamellar structure. Preferably, the peptide has the sequence shown in SEQ ID NOS: 5 or 6. In another aspect of this preferred embodiment, the mammal is a human.

The present invention also provides a method for inhibiting neural degeneration or promoting neurite outgrowth in neural tissue, comprising: contacting neural tissue susceptible to such degeneration with an effective neural degeneration-inhibiting amount of a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. Preferably, the peptide has the amino acid sequence shown in SEQ ID NOS: 5 or 6. Preferably, the administration is intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal or topical. Advantageously, the peptide is administered in a pharmaceutically acceptable carrier. In another aspect of this preferred embodiment, the peptide is enclosed in a lamellar structure. Preferably, the mammal is a human.

Still another embodiment of the invention is a method for treating neuropathic pain in a mammal in need thereof, comprising the step of administering to a mammal suffering from neuropathic pain an amount of a cyclic peptide effective to inhibit neuronal degeneration, wherein said peptide has between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. Preferably, the administering step is intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal, topical or oral. Advantageously, the peptide has the amino acid sequence shown in SEQ ID NOS: 5 or

6. Advantageously, the peptide is administered in a pharmaceutically acceptable carrier. In another aspect of this preferred embodiment, the peptide is enclosed in a lamellar structure. Preferably, the mammal is a human.

5 The present invention also provides a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; 10 and X_8 is a hydrophobic amino acid, for use in inducing myelination or inhibiting demyelination in a mammal. Preferably, the peptide has the sequence shown in SEQ ID NOS: 5 or 6.

Another embodiment of the invention is a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is 15 a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid for use in inhibiting neural degeneration or 20 promoting neurite outgrowth. Preferably, the peptide has the sequence shown in SEQ ID NOS: 5 or 6.

The present invention also provides a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, 25 phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid for use in treatment of neuropathic pain. Preferably, the peptide has the sequence shown in SEQ ID NOS: 5 or 6

30 Detailed Description of the Preferred Embodiment

The present invention provides cyclic saposin C-derived peptides having between about 11 and 25 amino acids, and including the consensus sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$ (SEQ ID NO: 4), wherein X_1 is a hydrophobic amino acid (alanine, leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid. In a preferred embodiment, the peptide has the sequence: cyclo-[LLDNNKTEKLY] (SEQ ID NO: 5) or cyclo-[LIDNNATEEIL] (SEQ ID NO: 6). Due to their constrained structures, these cyclic prosaposin peptidomimetics are significantly more resistant to enzymatic degradation and are capable of crossing the blood brain barrier to a greater extent than corresponding linear peptides. The cyclic peptides of the invention are equally as effective or more effective in stimulating neurite outgrowth than a known highly-active linear prosaposin peptidomimetic ("prosaptide") having the sequence TXLIDNNATEEILY, wherein X is D-alanine (SEQ ID NO: 7) (Example 1). Moreover, the cyclic peptides of the invention are more effective than prosaptide in preventing neural cell death *in vitro* (Example 2). These cyclic peptides lack free amino- and carboxy-terminal ends. Thus, they are more resistant to degradation *in vivo* by aminopeptidases and carboxypeptidases which degrade peptides from the amino-and carboxy-termini.

Cyclic saposin C-derived peptides comprising the active 11-mer region (SEQ ID NO: 4), and neurotrophic analogs thereof, have utility in promoting functional recovery after toxic, traumatic, ischemic, degenerative and inherited lesions to the peripheral and central nervous system. In addition, these peptides stimulate myelination and counteract the effects of demyelinating diseases. These peptides stimulate the outgrowth of neurons, promote myelination, promote neuroprotection and prevent programmed cell death in neuronal tissues in mammals, preferably humans. The peptides of the invention can also be used to treat various neuropathies including, but not limited to, motor, sensory, peripheral, taxol-induced and diabetic neuropathies. As used herein, a neuropathy is a functional disturbance or pathological change in the peripheral nervous system and is characterized clinically by sensory or motor neuron abnormalities. The peptides are also useful as analgesics, particularly for the

treatment of neuropathic pain in a mammal, preferably a human, which can develop days or months after a traumatic injury and is often long-lasting or chronic.

5 The second asparagine residue within the native prosaposin sequence (corresponding to second "N" in SEQ ID NO: 4) is known to be glycosylated with N-acetylglucosamine which may provide some resistance to proteolytic degradation. The synthetic modification of this asparagine residue within the instant non-native saposin C-derived peptides by standard methods (i.e. Merrifield synthesis) with various carbohydrates, preferably glucose, is also within the scope of the present invention.

10 One embodiment of the present invention is a method of facilitating neurite outgrowth or increased myelination in differentiated or undifferentiated neural cells by administering to the cells an effective, neurite outgrowth or myelin-facilitating amount of a saposin C-derived peptide encompassing the active cyclic 11-mer region shown in SEQ ID NO:4.

15 Saposin C-derived cyclic peptide analogs of the invention can differ from saposin C sequences or SEQ ID NOS: 4-6, for example, by replacement of one or more lysine and/or arginine residues; replacement of one or more tyrosine and/or phenylalanine residues, deletion of one or more phenylalanine residues and/or conservative replacement of one or more amino acids within the peptide. The replacement or deletion of lysine/arginine and tyrosine/phenylalanine residues will reduce the susceptibility of peptide degradation by trypsin and chymotrypsin, respectively. The cyclic neurotrophic and myelinotrophic peptide sequences of the invention preferably have up to about 50 amino acids; more preferably, up to about 30 amino acids; and most preferably, between about 11 and 25 amino acids and include therein the sequence shown in SEQ ID NO: 4.

20 Additional variations of these peptide sequences contemplated for use in the present invention include minor insertions, deletions and substitutions. For example, conservative amino acid replacements are contemplated. Such replacements are, for example, those that take place within a family of amino acids that are related in the chemical nature of their side chains. The families of amino acids include the basic charged amino acids (lysine, arginine, histidine); the acidic charged amino acids (aspartic acid, glutamic acid); the non-polar amino acids (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); the uncharged polar amino acids (glycine, asparagine, glutamine, cysteine, serine, threonine,

tyrosine); and the aromatic amino acids (phenylalanine, tryptophan and tyrosine). In particular, it is generally accepted that conservative amino acid replacements consisting of an isolated replacement of a leucine with an isoleucine or valine, or an aspartic acid with a glutamic acid, or a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not significantly affect the properties of the peptide. The cyclic saposin C-derived sequences including SEQ ID NO: 4 can be modified to attain various objectives such as increased activity and stability. Other amino acids can be present outside this consensus sequence including native saposin C sequence, conservative substitutions of these native sequences, or unrelated peptide sequences to achieve objectives such as increased binding, hydrophobicity, hydrophilicity and the like. Sequences outside the active neurotrophic region are not typically required for activity. Thus, in most instances, the subject peptide will be active regardless of the identity of these sequences. Again, any such peptide can be screened for such activity using the protocols described herein.

The ability of any such cyclic peptide to stimulate neurite outgrowth, prevent neural cell death, promote myelination, inhibit demyelination, alleviate neuropathic pain and treat sensory neuropathy can easily be determined by one of ordinary skill in the art using the procedures described in Examples 1-9. Methods for assaying the abilities of these peptides to promote myelination and to inhibit demyelination are set forth in Examples 3 and 4 hereinbelow.

A typical minimum amount of the peptides of the invention for the neurotrophic activity in cell growth medium is usually at least about 5 ng/ml. This amount or more of the cyclic synthetic peptides of the invention for *in vitro* use is contemplated. Typically, concentrations in the range of 0.1 µg/ml to about 10 µg/ml of these peptides will be used. Effective amounts for any particular tissue can be determined in accordance with Example 1.

The neural cells can be treated *in vitro* or *ex vivo* by directly administering the peptides of the invention to the cells. This can be done, for example, by culturing the cells in growth medium suitable for the particular cell type, followed by addition of the peptide to the medium. When the cells to be treated are *in vivo*, typically in a vertebrate, preferably a mammal, the composition can be administered by one of several techniques. Most preferably, the composition is injected directly into the blood or tissue in sufficient quantity to give the desired local concentration of peptide. In the peptides lacking lysine and arginine residues,

proteolytic degradation is reduced. The smaller peptides (i.e., 20-mer or less) will most likely cross the blood brain barrier and enter the central nervous system for treatment of CNS disorders (see Banks et al., *Peptides*, 13:1289-1294, 1992).

5 The peptides of the invention may also be esterified with fatty acids to form peptide fatty acid esters using conventional acid-catalyzed esterification. Alternatively, the last amino acid added in the synthetic procedure is itself a commercially available esterified amino acid which obviates the need for the esterification reaction. Fatty acids contemplated for use in formation of peptide esters include lauric, myristic, palmitic, stearic, oleic and linoleic.

10 The subject peptides may also be acetylated by inclusion of commercially available acetylated lysine, arginine or asparagine residues during the synthetic procedure. These modified peptides retain the activity of the parent compound. Such modifications will facilitate the ability of the peptide to cross the blood brain barrier due to increased hydrophobicity.

15 For treatment of neural disorders, direct intracranial injection or injection into the cerebrospinal fluid may also be used in sufficient quantities to give the desired local concentration of neurotrophin. In both cases, a pharmaceutically acceptable injectable carrier is used. Such carriers include, for example, phosphate buffered saline and Ringer's solution. Alternatively, the composition can be administered to peripheral neural tissue by direct local injection or by systemic administration. Various conventional modes of administration are
20 contemplated, including intravenous, intracerebrospinal, intramuscular, intradermal, subcutaneous, intracranial, intranasal, epidural, topical and oral. For use as an analgesic, administration by direct intramuscular or intravenous injection is preferred.

The peptide compositions of the invention can be packaged and administered in unit dosage form, such as an injectable composition or local preparation in a dosage amount
25 equivalent to the daily dosage administered to a patient or as a controlled release composition. A septum sealed vial containing a daily dose of the active ingredient in either PBS or in lyophilized form is an example of a unit dosage. Appropriate daily systemic dosages of the peptides of the invention based on the body weight of the vertebrate for treatment of neural diseases, demyelination or as an analgesic in general or for treatment of
30 neuropathic pain are in the range of from about 0.01 to about 10,000 $\mu\text{g/kg}$. More preferably, daily systemic dosages are between about 0.1 and 1,000 $\mu\text{g/kg}$. Most preferably,

daily systemic dosages are between about 10 and 100 $\mu\text{g/kg}$. Thus, for the typical 70 kg human, dosages can be between about 0.7 and 700,000 μg daily; more preferably between about 7 and 70,000 μg daily; and most preferably between about 700 and 7,000 $\mu\text{g/kg}$. Daily dosages of locally administered material will be about an order of magnitude less. Oral administration is also contemplated.

In one preferred embodiment of the invention, the neurotrophic peptides are administered locally to neural cells *in vivo* by implantation thereof. For example, polylactic acid, polygalactic acid, regenerated collagen, multilamellar liposomes and many other conventional depot formulations is expressly contemplated in the present invention. Infusion pumps, matrix entrapment systems and combination with transdermal delivery devices are also contemplated. The peptides may also be encapsulated within a polyethylene glycol conformal coating as described in U.S. Patent No. 5,529,914 prior to implantation.

The neurotrophic peptides of the invention may also be enclosed in micelles or liposomes. Liposome encapsulation technology is well known. Liposomes may be targeted to specific tissue, such as neural tissue, through the use of receptors, ligands or antibodies capable of binding the targeted tissue. The preparation of these formulations is well known in the art (Radin et al., *Meth. Enzymol.*, 98:613-618, 1983).

There are currently no commercially available pharmaceuticals capable of promoting full functional regeneration and restoration of the structural integrity of neural systems. This is particularly true of the CNS. Regeneration of peripheral nerves through the use of neurotrophic factors is within the scope of the invention. Moreover, neurotrophic factors can be therapeutically useful in the treatment of neurodegenerative diseases associated with the degeneration of neural populations or specific areas of the brain. The principal cause of Parkinson's disease is the degeneration of dopaminergic neurons of the substantia nigra. Since antibodies against prosaposin immunohistochemically stain the dopaminergic neurons of the substantia nigra in human brain sections, the neurotrophic peptides of the invention may be therapeutically useful in the treatment of Parkinson's disease. Retinal neuropathy, an ocular neurodegenerative disorder leading to loss of vision in the elderly, is also treatable using the peptides of the invention.

It has long been believed that in order to reach neuronal populations in the brain, neurotrophic factors would have to be administered intracerebrally since these proteins do not

cross the blood brain barrier. U.S. Patent No.5,571,787 discloses that an iodinated neurotrophic 18-mer fragment derived from saposin C crosses the blood brain barrier. Peptides of the present invention having up to about 22 amino acids will also cross this barrier and can thus be administered intravenously, with greater transport occurring for shorter peptides. Other neuronal populations, such as motor neurons, can also be treated by intravenous injection, although direct injection into the cerebrospinal fluid is also envisioned as an alternate route.

Cells may be treated to facilitate myelin formation or to prevent demyelination in the manner described above *in vivo*, *ex vivo* or *in vitro*. Diseases resulting in demyelination of nerve fibers including MS, acute disseminated leukoencephalitis, progressive multifocal leukoencephalitis, metachromatic leukodystrophy and adrenal leukodystrophy can be slowed or halted by administration of the neurotrophic peptides of the invention to the cells affected by the disease. Reversal of demyelination diseases or other neural damage is also contemplated.

The compositions of the present invention can be used *in vitro* as research tools for studying the effects of neurotrophic factors and myelin facilitating materials. However, more practically, they have an immediate use as laboratory reagents and components of cell growth media for facilitating growth and maintaining neural cells *in vitro*.

The peptides of the invention are synthesized on a solid support using established methods and Fmoc chemistry using an automated solid-phase protocol well known in the art (Traeciak et al., *Tetrahedron Lett.* 33:4557-4561, 1992) on a Protein Technologies Symphony peptide synthesizer. The cyclized peptides were cleaved from the solid support using TFA/water/triisopropylsilane (95:2.5:2.5). The peptides were purified using reverse phase HPLC on a C-18 column eluting with 0.3% trifluoroacetic acid (TFA) in acetonitrile. Mass spectral analysis of the peptides shown in SEQ ID NOS: 5 (peptide A) and 6 (peptide B) confirmed that cyclic peptides had been synthesized [SEQ ID NO: 5: MH⁺1333 (expected), 1333 (observed); SEQ ID NO: 6 MH⁺1127 (expected), 1127 (observed)].

The following examples are illustrative and are not intended to limit the scope of the present invention.

Example 1

Stimulation of neurite outgrowth *in vitro*

NS20Y neuroblastoma cells were grown in DMEM containing 10% fetal calf serum (FCS). Cells were removed with trypsin and plated in 30 mm petri dishes onto glass coverslips. After 20-24 hours, the medium was replaced with 2 ml DMEM containing 0.5% FCS plus various concentrations of the peptide A, peptide B or TX14(A). Cells were cultured for an additional 24 hours, washed with PBS and fixed with Bouin's solution (saturated aqueous picric acid/formalin/acetic acid 15:5:1) for 30 minutes. Fixative was removed with PBS and neurite outgrowth was scored under a phase contrast microscope. Cells exhibiting one or more clearly defined neurites equal to or longer than one cell diameter were scored as positive. At least 200 cells were scored in different portions of each dish to determine the percentage of neurite bearing cells and assays were performed in duplicate.

All three peptides induced neurite outgrowth in NS20Y cells. The $T_{1/2}$ (ED_{50}), which is the concentration of peptide resulting in 50% increased neurite outgrowth, was 1.0 ng/ml for TX14(A) and for peptide B. The $T_{1/2}$ for peptide A was 0.6 ng/ml. Thus, peptide B was as effective as TX14(A), while peptide A was more effective than TX14(A). This indicates that the cyclized peptides of the present invention have excellent activity in comparison to an established standard "prosaptide".

Example 2

Prevention of cell death *in vitro*

NS20Y cells were plated as described in Example 1 and grown on glass coverslips in 0.5% fetal bovine serum for 2 days in the presence or absence of TX14(A), peptide A or peptide B. Media was removed and 0.2% trypan blue in PBS was added to each well. Blue-staining dead cells were scored as a percentage of the total on an inverted microscope, counting 400 cells in four areas of each well. The average error of duplicates was 5%. TX14(A), peptide A and peptide B reduced the number of trypan blue-positive (dead) cells. The $T_{1/2}$ (ED_{50}) for prevention of neural cell death for TX14(A) was 1.0 ng/ml. Peptides A and B were more potent, with $T_{1/2}$ values of 0.6 ng/ml and 0.8 ng/ml, respectively. This indicates that the cyclic peptides have exceptional activity in rescuing neural cells from programmed cell death in comparison to a standard linear "prosaptide".

Example 3

Ex vivo myelination assay

Newborn mouse cerebellar explants are prepared according to Satomi (*Zool. Sci.*, 9:127-137, 1992). Neurite outgrowth and myelination are observed over 22 days in culture, during the period when the newborn mouse cerebellum normally undergoes neuronal differentiation and myelination begins. A cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4 (10 µg/ml) is added on the second day after preparation of the explants (three control and three treated explants), and outgrowth of neurites and myelination is assessed under a bright field microscope with a video camera. On the eighth day, cultures containing the peptides are thinner and more spread out than control cultures. On day 15, peptide-treated cultures contain many cells with long projections at the periphery of the explant which are less prominent in untreated control cultures. Peptide-treated cultures contain significantly more myelinated axons in the subcortical white matter at 22 days compared to control explants. Thus, the peptides of the invention induce increased myelination in differentiating cerebellum *ex vivo*.

Example 4

Prevention of demyelination

The prevention of Schwann cell death is correlated with prevention of demyelination. Schwann cells contain an extensive myelin sheath. The addition of a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4 to Schwann cells in culture reduces Schwann cell death in a dose-dependent manner and stimulates the incorporation of sulfatides, myelin-specific lipids, into Schwann cells. This indicates that the cyclic peptides of the invention can prevent demyelination due to Schwann cell death.

Example 5

Use of peptides in treating traumatic ischemic CNS lesions

Humans with traumatic lesions to the spinal cord receive intracerebrospinal or direct injection of about 100 µg/ml of a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4 in a sterile saline solution or in depot form to enable slow, continuous release of the peptide at the lesion site. Improvement is assessed by gain of motor nerve function (i.e. increased limb movement). Treatments continue until no further improvement occurs.

Example 6

Use of peptides in treating demyelination disorders

Patients diagnosed with early stage MS are given a cyclic saposin C-derived peptide having between 11 and 25 amino acids, and containing the sequence shown in SEQ ID NO: 4, by direct intravenous injection into the cerebrospinal fluid using the same dose range as in Example 3. Dosages are repeated daily every 2-5 days, or weekly and improvement in muscle strength, musculoskeletal coordination and myelination (as determined by MRI) is observed.

Example 7

Alleviation of neuropathic pain in Chung model rats

This example describes the effects of bolus intrathecal injection of a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4 in the Chung experimental model of peripheral neuropathic pain. Each peptide is chemically synthesized, purified, dissolved in sterile PBS and buffered to neutral pH. The surgical procedure previously described by Kim et al. (*Pain*, 50:355, 1992) is performed on male rats to induce an allodynic state. A spinal catheter is introduced two weeks after surgery. Five days later, the peptides are administered at 0.007, 0.07 and 0.7 µg/rat. Pressure thresholds are then determined using calibrated von Frey hairs. The longer the time taken for an animal to withdraw the paw in response to applied pressure, the less severe the neuropathic pain. The peptides significantly increase the threshold pressure, indicating a significant alleviation of neuropathic pain.

Example 8

Treatment of sensory neuropathy

Mice are administered taxol in order to induce sensory neuropathy. Taxol-treated mice are administered 50 µg/kg, 100 µg/kg or 250 µg/kg of a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4. The loss of thermal sensation is measured using a Hargreaves sensory testing apparatus as an indicator of sensory neuropathy. Each of the three doses of peptide is effective in preventing or retarding loss of thermal sensation in taxol-treated mice. Thus, the synthetic saposin C-derived peptides of the invention effectively inhibit sensory neuropathy.

Example 9

Alleviation of neuropathic pain in diabetic rats

This example describes the effects of kg of a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4 by intraperitoneal administration in a rat model of diabetic neuropathy.

5 Rats are made diabetic by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight, freshly dissolved in 0.9% sterile saline) to ablate pancreatic β cells and induce insulin deficiency as described by Calcutt et al. (*Pain*, 68:293-299, 1996). Two days later, diabetes is confirmed in streptozotocin-injected rats by measuring blood glucose levels. Streptozotocin-injected animals with a blood glucose concentration below 15 mmol/l were excluded from subsequent studies, according to the commonly accepted definition of non-
10 fasting hyperglycemia in studies of diabetes in rats.

Both diabetic and control rats are studied at 8 weeks by analyzing the behavioral response to the noxious chemical formalin as an indicator of allodynia (Calcutt et al., *supra*, 1996). Briefly, rats receive a subcutaneous injection of freshly-prepared formalin (50 μ l of 0.5% solution in sterile saline) into the dorsal surface of the right hind paw. This
15 concentration of formalin induces sub-maximal behavioral responses in control rats and allows detection of hyperalgesia in diabetic rats during phases Q and 2 (Calcutt et al., *Eur. J. Pharmacol.*, 285:189-197, 1995). Animals are transferred to an observation chamber constructed to allow continuous visualization of the paws. The number of flinches during one minute periods is counted at 5 minute intervals for the next 60 minutes by an observer who is
20 unaware of the treatment group of each animal. Phase 1 is defined as the initial measurement of flinching (1-2 and 5-6 minutes post injection); the Q (quiescent) phase as the measurements made at 10-11, 15-16 and 20-21 minutes; and Phase 2 as all subsequent measurements post-injection, as previously defined for studies of diabetic rats (see, for example, Malmberg et al., *Neurosci. Lett.*, 161:45-48, 1993). Comparisons of activity during
25 each phase are made by summing the flinches at measurement points within the phase. diabetic rats give an abnormal flinch response.

Diabetic rats are divided in two groups of four animals each which are administered saline or a cyclic saposin C-derived peptide having between 11 and 25 amino acids and containing the sequence shown in SEQ ID NO: 4, respectively. Two hours before treatment
30 with 0.5% formalin, the diabetic rats are treated with saline or 200 μ g/kg peptide using intraperitoneal administration. Administration of peptide completely prevents the abnormal

flinch response in Phase 1 and ameliorates the response in Phase 2 by 70%. Thus, parenteral administration of peptide alleviates the pain from formalin injection and improves motor neuron function in a rat model of painful diabetic neuropathy.

SEQUENCE LISTING

(I) GENERAL INFORMATION:

(i) APPLICANT: MYELOS CORPORATION

(ii) TITLE OF INVENTION: CYCLIC ANALGESIC AND NEUROTROPHIC PEPTIDES

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

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- (C) CITY: Newport Beach
- (D) STATE: CA
- (E) COUNTRY: U.S.A.
- (F) ZIP: 92660

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: Windows
- (D) SOFTWARE: FastSEQ for Windows Version 2.0b

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 60/098,359
- (B) FILING DATE: 28-AUG-1998

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Bartfeld, Neil S
- (B) REGISTRATION NUMBER: 39,901
- (C) REFERENCE/DOCKET NUMBER: MYELOS.014PR

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 619-235-8550
- (B) TELEFAX: 619-235-0176
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Glu Phe Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys
1 5 10 15
Thr Glu Lys Glu Lys
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Tyr Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys Glu
1 5 10 15
Ile Leu

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Ile Asp Asn Asn Lys Thr Glu Lys Glu Ile Leu
 1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Other
- (B) LOCATION: 1...2
- (D) OTHER INFORMATION: A,L,I,V,Y,W,F OR M

- (A) NAME/KEY: Other
- (B) LOCATION: 3...3
- (D) OTHER INFORMATION: D,E,K OR R

- (A) NAME/KEY: Other
- (B) LOCATION: 6...6
- (D) OTHER INFORMATION: ANY AMINO ACID

- (A) NAME/KEY: Other
- (B) LOCATION: 8...8
- (D) OTHER INFORMATION: D OR E

- (A) NAME/KEY: Other
- (B) LOCATION: 9...9
- (D) OTHER INFORMATION: ANY AMINO ACID

- (A) NAME/KEY: Other
- (B) LOCATION: 10...11
- (D) OTHER INFORMATION: A,L,I,V,Y,W,F OR M

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Xaa Xaa Xaa Asn Asn Xaa Thr Xaa Xaa Xaa Xaa
 1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: cyclic

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Leu Asp Asn Asn Lys Thr Glu Lys Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: cyclic

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Ile Asp Asn Asn Ala Thr Glu Glu Ile Leu
1 5 10

WHAT IS CLAIMED IS:

1. A cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid.
2. The peptide of Claim 1, wherein the peptide has the amino acid sequence shown in SEQ ID NO: 5 or 6.
3. A composition comprising the peptide of Claim 1, in a septum sealed vial.
4. A composition comprising the peptide of Claim 1, formulated with a controlled release material.
5. A composition comprising the peptide of Claim 1, in lyophilized form.
6. A composition comprising the peptide of Claim 1, in liposomal form.
7. A composition comprising the peptide of Claim 1, in a form suitable for topical administration.
8. A composition comprising the peptide of Claim 1, in unit dosage form.
9. A method for inducing myelination or inhibiting demyelination in a mammal, comprising:
administering to a mammal afflicted with demyelination a pharmaceutically effective demyelination inhibiting amount of a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid.
10. The method of Claim 9, wherein said demyelination is due to multiple sclerosis, ischemic injury or traumatic injury.

11. The method of Claim 9, wherein said administration is selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal and topical.
12. The method of Claim 9, wherein said peptide is administered in a pharmaceutically acceptable carrier.
13. The method of Claim 9, wherein said peptide is enclosed in a lamellar structure.
14. The method of Claim 9, wherein said peptide has the sequence shown in SEQ ID NOS: 5 or 6.
15. The method of Claim 9, wherein said mammal is a human.
16. A method for inhibiting neural degeneration or promoting neurite outgrowth in neural tissue, comprising:
contacting neural tissue susceptible to such degeneration with an effective neural degeneration-inhibiting amount of a cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid.
17. The method of Claim 16, wherein said administration is selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal and topical.
18. The method of Claim 16, wherein said peptide is administered in a pharmaceutically acceptable carrier.
19. The method of Claim 16, wherein said peptide is enclosed in a lamellar structure.
20. The method of Claim 16, wherein said peptide has the amino acid sequence shown in SEQ ID NOS: 5 or 6.
21. The method of Claim 16, wherein said mammal is a human.
22. A method for treating neuropathic pain in a mammal in need thereof, comprising the step of administering to a mammal suffering from a neurodegenerative disease an amount of a cyclic peptide effective to treat neuropathic pain, wherein said peptide contains between about 11 and 25 amino acids, and includes the sequence

$X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid.

23. The method of Claim 22, wherein said administering step is selected from the group consisting of intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal, topical and oral.
24. The method of Claim 22, wherein said peptide is administered in a pharmaceutically acceptable carrier.
25. The method of Claim 22, wherein said peptide is enclosed in a lamellar structure
26. The method of Claim 22, wherein said peptide has the amino acid sequence shown in SEQ ID NOS: 5 or 6.
27. The method of Claim 22, wherein said mammal is a human.
28. A cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid, for use in inducing myelination or inhibiting demyelination in a mammal.
29. The peptide of Claim 28, wherein said peptide has the sequence shown in SEQ ID NOS: 5 or 6.
30. A cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid for use in inhibiting neural degeneration or promoting neurite outgrowth.

31. The peptide of Claim 30, wherein said peptide has the sequence shown in SEQ ID NOS: 5 or 6.

5 32. A cyclic peptide having between about 11 and 25 amino acids, and including the sequence $X_1X_2X_3NNX_4TX_5X_6X_7X_8$, wherein X_1 is a hydrophobic amino acid (leucine, alanine, isoleucine, valine, tyrosine, tryptophan, phenylalanine or methionine); X_2 is a hydrophobic amino acid, X_3 is aspartic acid, glutamic acid, lysine or arginine; N is asparagine, X_4 is any amino acid; T is threonine; X_5 is glutamic acid or aspartic acid; X_6 is any amino acid, X_7 is a hydrophobic amino acid; and X_8 is a hydrophobic amino acid for use in treatment of neuropathic pain.

10 33. The peptide of Claim 32, wherein said peptide has the sequence shown in SEQ ID NOS: 5 or 6.

INTERNATIONAL SEARCH REPORT

Inter. Appl. No.

PCT/US 99/19378

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/475 A61K38/18 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| Y | WO 97 32895 A (BRIEN JOHN S O ;UNIV CALIFORNIA (US)) 12 September 1997 (1997-09-12) abstract | 1-33 |
| Y | KOTANI E A: "A hydrophilic peptide comprising 18 amino acid residues of the prosaposin sequence has neurotrophic activity in vitro and in vivo" JOURNAL OF NEUROCHEMISTRY, US, NEW YORK, NY, vol. 5, no. 66, 1 January 1996 (1996-01-01), pages 2197-2200, XP002077621 ISSN: 0022-3042 the whole document | 1-33 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

2 February 2000

Date of mailing of the international search report

14/02/2000

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/US 99/19378

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| Y | <p>O'BRIEN E A: "Identification of the neurotrophic factor sequence of prosaposin" FASEB JOURNAL, US, FED. OF AMERICAN SOC. FOR EXPERIMENTAL BIOLOGY, BETHESDA, MD, no. 9, 1 May 1995 (1995-05-01), pages 681-685, XP002077620 ISSN: 0892-6638 cited in the application the whole document</p> <p>---</p> | 1-33 |
| Y | <p>LIEPINSH E.A.: "Saposin fold revealed by the NMR structure of NK-lysin" NATURE STRUCTURAL BIOLOGY, vol. 4, no. 10, October 1997 (1997-10), pages 793-795, XP000867792 cited in the application See especially page 795, column 1</p> <p>-----</p> | 1-33 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/19378

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 9-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

INTERNATIONAL SEARCH REPORT

information on patent family members

Interr. .nal Application No
PCT/US 99/19378

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO 9732895 A | 12-09-1997 | AU 2328697 A | 22-09-1997 |
| | | CA 2248139 A | 12-09-1997 |
| | | CZ 9802839 A | 12-05-1999 |
| | | EP 0929569 A | 21-07-1999 |
| | | NO 984093 A | 04-11-1998 |
| | | NZ 331757 A | 28-10-1999 |
| | | PL 328701 A | 15-02-1999 |

Form PCT/ISA/210 (patent family annex) (July 1992)

BNSDOCID: <WO____0012553A1_I_>

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